

Single-cell analysis of yeast (*Saccharomyces cerevisiae*) using hydrogel encapsulation

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Background

Space radiation poses a major health risk to astronauts. To fulfill NASA's mission of exploration beyond Earth, the biological effects of Galactic Cosmic Radiation must be investigated to elucidate cellular damage mechanisms and inform countermeasure protocols to safely bring humans beyond Earth's magnetosphere.

Budding yeast (*Saccharomyces cerevisiae*) are commonly used in experiments as a model organism for studying the effects of radiation on eukaryotes.

We are using a novel method of microencapsulation in hydrogel particles PicoShells (Ng et. al 2022, van Zee et al. 2022) to enable analysis of the distribution of radiation-induced damage among yeast cells at the single-cell level, in high throughput.

Methods

Yeast encapsulated in PicoShells by DiCarlo Lab at UCLA and shipped to NASA Ames Research Center

Encapsulated yeast cultured in YPD medium, harvested at regular timepoints, fixed in ethanol and stained with Propidium iodide

Samples filtered and numerated using Guava easyCyte 2.0, and by fluorescence microscopy

Analyzed flow cytometry data using flowcore package in RStudio

Results

Yeast growth time series

Purpose: To evaluate our ability to track growth of yeast encapsulated in PicoShells using flow cytometry and microscopy

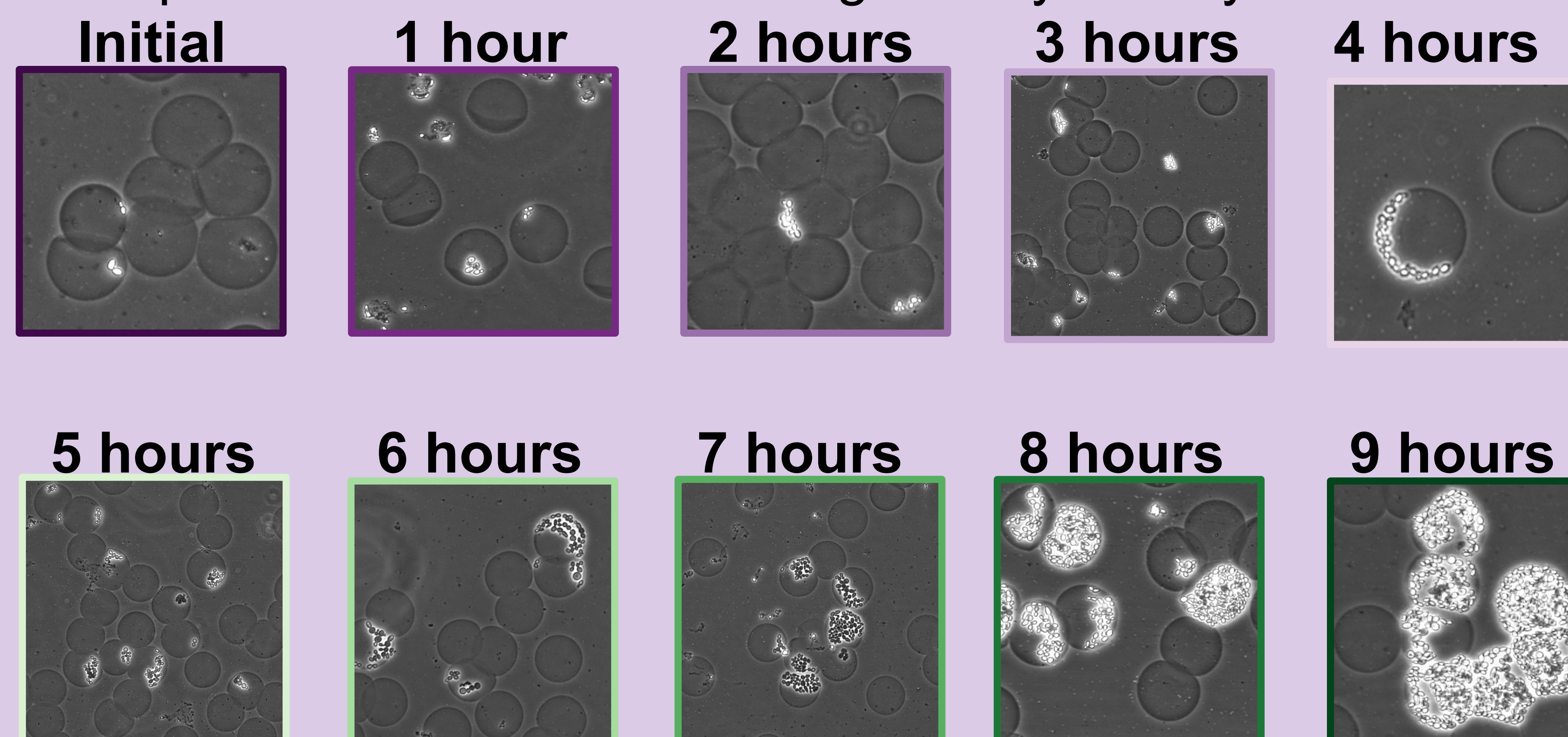


Figure 1. Yeast growth encapsulated in PicoShells over 9 hours cultured in YPD at 30 C

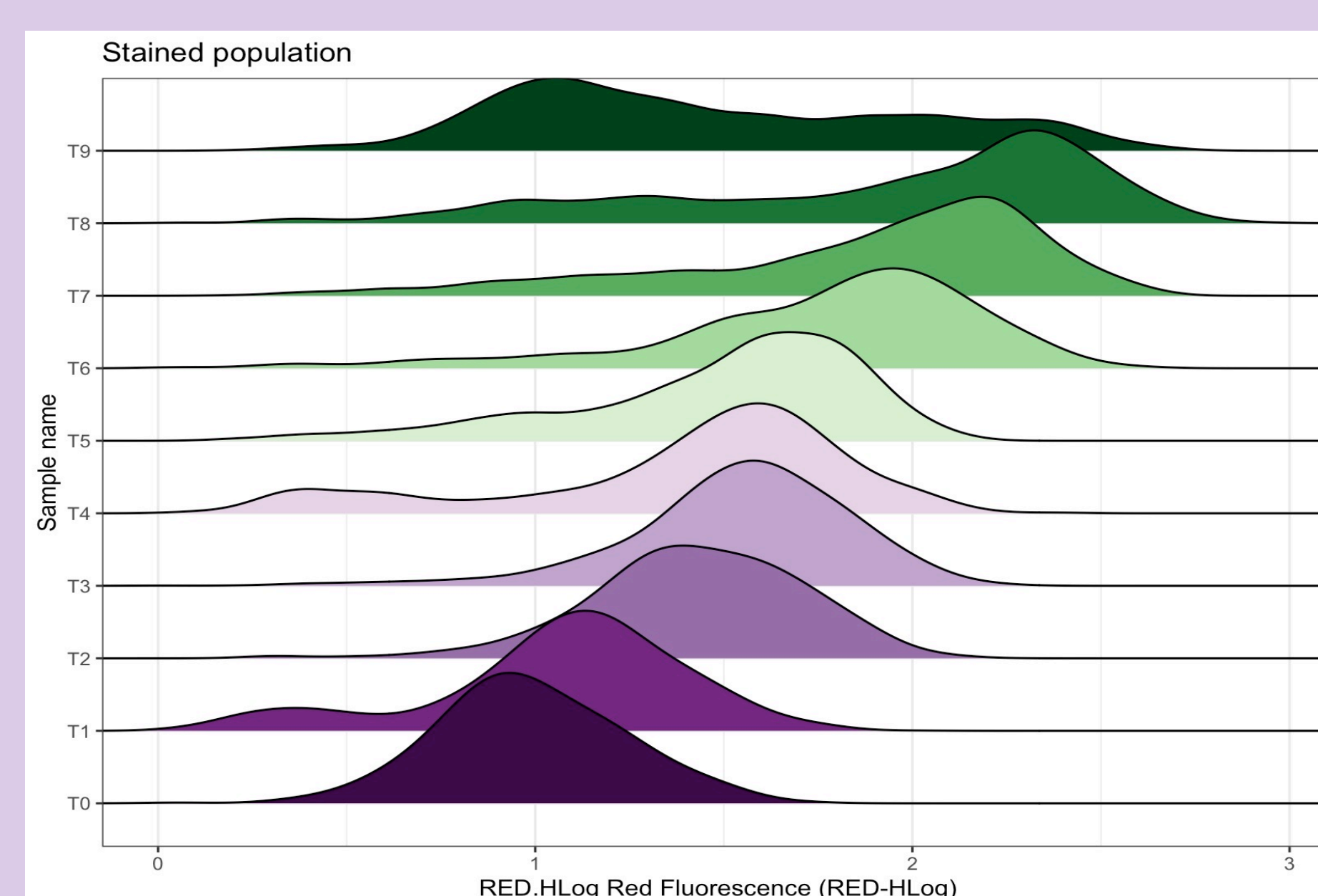


Figure 2. Distribution of flow cytometry events at a wavelength of 488 nm (red fluorescence) of PicoShell encapsulated yeast cultured in YPD at 30 C over 9 hours

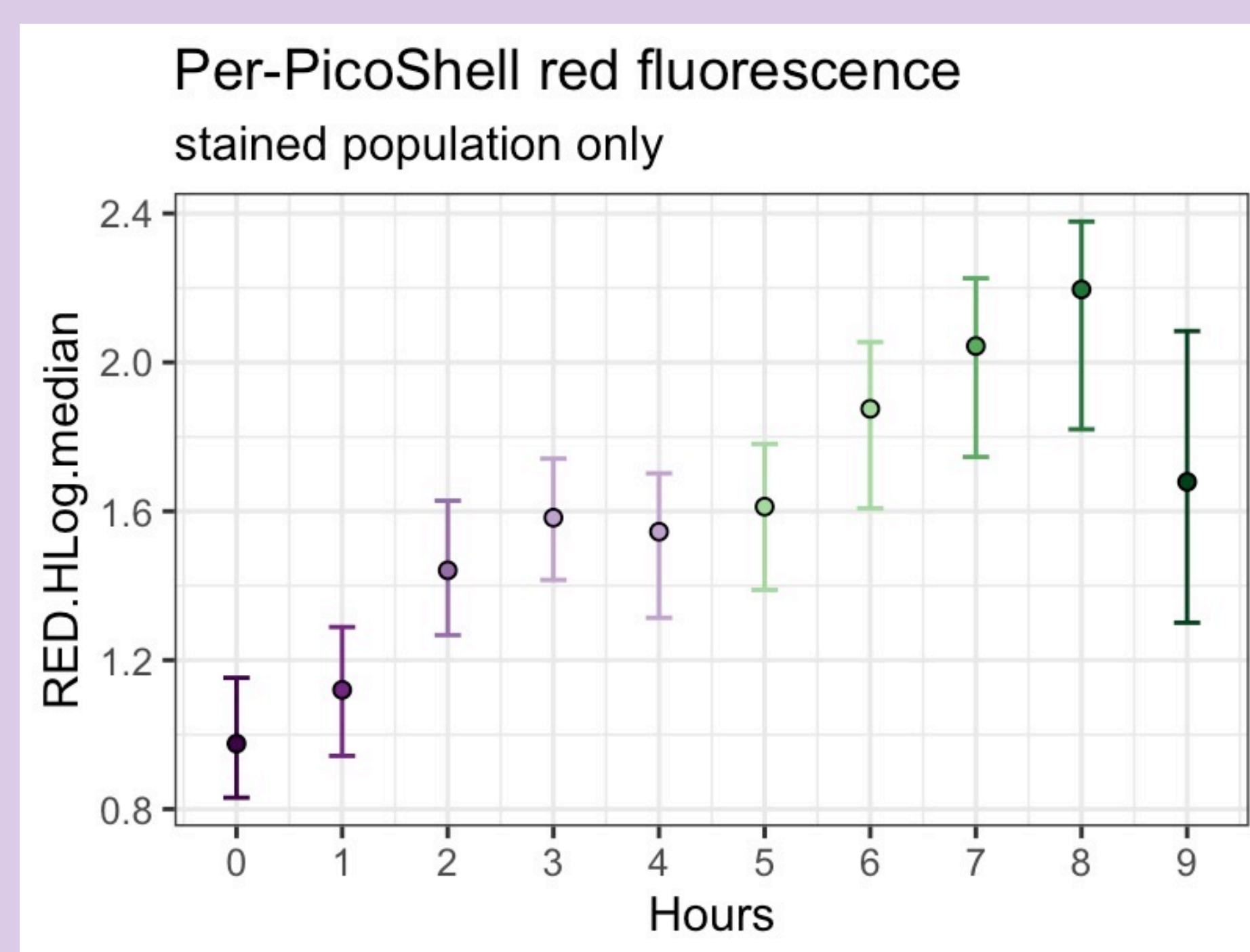


Figure 3. Median, first quartile and third quartile of red fluorescence per PicoShells from Figure 2, dotted line shows fitted growth rate of fluorescence per PicoShell, doubling time calculated to be 5.20 hours

Conclusions

- Yeast cells encapsulated in PicoShells can successfully be fixed with ethanol and stained with Propidium iodide
- We found the doubling time of per PicoShell fluorescence to be higher than that of yeast in YPD ~2.5 hours, likely indicating that fluorescence does not correlate with number of yeast cells
- Decrease in fluorescence at approximately 8 hours is potentially due to bursting of PicoShells from yeast growth and release of individual yeast cells

Future Directions

- Develop live and dead cell viability procedure
- Assess variability in yeast colony sizes
- Test different staining compounds and procedures
- Treat yeast with various radiation treatments

Acknowledgements

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References

1. Ng, Simon, Cayden Williamson, Mark van Zee, Dino Di Carlo, and Sergio R. Santa Maria. "Enabling Clonal Analyses of Yeast in Outer Space by Encapsulation and Desiccation in Hollow Microparticles." *Life* 12, no. 8 (August 2022): 1168. <https://doi.org/10.3390/life12081168>.
2. Zee, Mark van, Joseph de Rutte, Rose Rumyan, Cayden Williamson, Trevor Burnes, Randor Radakovits, Andrew Sonico Eugenio, et al. "High-Throughput Selection of Cells Based on Accumulated Growth and Division Using PicoShell Particles." *Proceedings of the National Academy of Sciences* 119, no. 4 (January 25, 2022): e2109430119. <https://doi.org/10.1073/pnas.2109430119>.